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KARYOKINESIS IN THE ROOT TIPS OF ALLIUM CEPA.[1]

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(WITH PLATES XXI AND XXII)

FOR several years the writer has desired to study in detail the formation of the achromatic spindle in the root tips of *Allium Cepa*, having frequently seen interesting figures in the early stages of division while investigating the subject of centrospheres in this plant. Especially was the desire increased when the interesting series of papers appeared from the Bonn Botanical Institute[2] dealing especially with the origin of the nuclear spindle. Accordingly a set of preparations was made, the material being killed in several fixing fluids, and stained in various ways, so that any irregularity due to technique might be eliminated. Flemming's weaker and stronger fluid and chrom-acetic acid seemed to give the best results, although several others worked fairly well. So far as the appearance of the spindle was concerned there did not seem to be any great difference in the effect produced by the several fluids. Chrom-acetic acid is without doubt the best for general purposes, as it preserves the structures of the dividing nucleus just as faithfully as Flemming's, and does not interfere with the action of the stains used.

[1] Contributions from the botanical laboratory of Ohio State University. III.

[2] Jahrbücher für wiss. Bot. 30: 159–422. 1897.

The proportions were as follows: Chromic acid, 0.8^{cc}; acetic acid, 0.5^{cc}; water, 99.0^{cc}. The combinations of stains giving the best results were anilin-safranin and gentian-violet; iron-alum-haematoxylin; and anilin-safranin and iron-alum-haematoxylin.

ANILIN-SAFRANIN, GENTIAN-VIOLET.

1. Anilin-safranin alcoholic (50 per cent.) solution prepared by combining equal parts of anilin water, and saturated alcoholic (95 per cent.) solution of safranin.

2. Gentian-violet 2 per cent. aqueous solution. Stain from two to four hours in the safranin, and from two to four minutes in the gentian-violet. The slides must be taken through the alcohols quite rapidly or the stain will be lost.

HEIDENHAIN'S IRON-ALUM-HAEMATOXYLIN.

1. Ammonio-sulphate of iron 2 per cent. aqueous solution.

2. Haematoxylin, a ½ per cent. solution obtained by dissolving in hot water.

Keep the sections from two to four hours in the iron-alum, and then from eight to twelve hours in the haematoxylin, afterwards taking out the excess of stain with the iron-alum until the sections are of the proper color.

ANILIN-SAFRANIN, IRON-ALUM-HAEMATOXYLIN.

This was by far the best combination used, bringing out with remarkable distinctness chromatin network, chromosomes, nucleoli, spindles and centrospheres. The centrosomes were especially distinct in some pollen mother cells of *Sagittaria variabilis*, showing as large, black, spherical granules at the poles of the spindle. The sections are stained in the usual way in the anilin-safranin for two or three hours, and then carried through the iron-alum-haematoxylin in the same manner as when this combination is used alone. This method, although tedious, will amply repay in results for the long time necessary for the staining. The combination is improved a little, perhaps, by staining for two minutes after the anilin-safranin in gentian-violet.

The material was imbedded in paraffin, sectioned from 10–18 μ thick and stained on the slide.

The root tips of *Allium Cepa* L. are very favorable objects for the study of karyokinesis, and in making a critical investigation of the structures and activities of the cell during division

it was thought best to take some such common object, which could be followed easily in the class room, and tested as to its accuracy. Accounts and figures of karyokinesis in plant cells are very scarce, and the so-called diagrammatic or schematic figures and descriptions given in most of the text-books are but a poor guide for the student and young investigator. For these reasons a rather complete account of the whole process has been given.

The most typical resting nuclei occur some distance back of the tip beyond the actively dividing region. Here, in good stained material, the nuclei usually have one or two large nucleoli and a very distinct chromatin network, with large irregular chromatin granules, which usually appear at the crossings of the meshes (*fig. 1*). In these cells there are large vacuoles, and it is rarely that the cytoplasmic contents or the centrospheres show to advantage. However, if one goes near the tip, in the actively dividing region, it is easy to find cells showing all the various cell organs usually present. The cells mostly divide in but one plane by transverse walls, and at the upper or lower side of the nucleus there is usually a depression in which two small bodies lie (*fig. 2*). The presence of this depression, and two characteristic bodies in it leave but little doubt as to their nature. They are to all intents and purposes centrospheres. Just at the time when the nucleus begins to divide it generally stains much deeper, and around it may be seen radiating streams of cytoplasm (*fig. 3*). While the nucleus is in this condition the finer chromatin threads disappear. Just how this disappearance takes place it is not easy to tell. The finer meshes seem to be drawn into the coarser threads, or if this is not the case the whole thread shortens and thickens, thus becoming more evident, and also giving the appearance that there are fewer threads present. While this process is going on the centrospheres separate and take up their positions on opposite sides of the nucleus, being closely applied to the nuclear membrane (*fig. 4*). As the chromatin thread continues to shorten and thicken the incept[3] of the

[3] The word *incept* is used as the equivalent of the German *Anlage*.

achromatic spindle makes its appearance. This arises as two flattened, dome-shaped prominences on opposite sides of the nucleus. These seem to inclose the nucleus completely, and at their summits can usually be seen two spherical bodies, the centrospheres, each with a dark center, the centrosome, around which there is a series of cytoplasmic radiations (*figs. 5, 8, 11*). Sometimes there is an outer granular zone near the limit of the radiations (*figs. 8, 24*). Thus it seems that the spindle originates from the two opposite centrospheres. The spindle usually arises on the two flattened sides of the nucleus (*figs. 5, 10, 12*), but sometimes it originates on the ends of the long axis of the nucleus. It is nearly always very much rounded and flattened at first, except in cases of long narrow cells, in which it seems to be pointed from the very first. In the younger stages the radiations are often not very marked, in other cases they are very distinct and very thick but few in number. In case the spindle is formed on the ends of the long axis of the nucleus it cannot be seen as early as usual, since it then lies very close to the nucleus (*fig. 7*). Although the centrospheres generally separate quite early and take their position on opposite sides of the nucleus, they may sometimes be considerably delayed. *Fig. 9* seems to be such a case, where the chromatin band is well formed but the two centrospheres are still close together and their centrosomes have begun to divide. This figure may be explained by supposing a late separation of the centrospheres and a precocious division of the centrosomes. There are beautiful, delicate radiations passing out into the cytoplasm. In some cells the incept of the spindle remains dome-shaped and very much flattened for a long time, and frequently no bodies can be seen which look like centrosomes. It need not be implied, however, that centrosomes are not present in such cases. In cells of about the same age the spindles are often becoming pointed and show a centrosphere in close contact with the spindle fibers, and having well developed radiations around the poles (*figs. 11, 13*). In *fig. 10* there is a system of streams of cytoplasm passing out from the young spindle to the cell wall. These are no doubt ordinary

delicate streams of cytoplasm and are of the same nature as those shown in *fig. 3*. They have nothing to do directly with the formation of the spindle. The incept of the spindle is very sharply differentiated from the surrounding cytoplasm and the space between it and the nuclear membrane appears very clear and transparent, like the achromatin of the nucleus.

After the chromatin band has become considerably thickened it loops up into sixteen definite loops, the heads of which, in typical cases, point toward the two poles (*figs. 13, 14*). The loops, however, do not always seem to have this position in relation to the poles, as is shown by *figs. 15* and *16*. When one looks down from one pole nothing is seen of the nuclear spindle (*fig. 14*). The dome-shaped spindle gradually extends outward and becomes pointed, until the time of the breaking of the chromatin coil into a definite number of chromosomes, accompanied by the disappearance of the nuclear membrane (*figs. 15–20*). In these stages the centrospheres become more prominent, probably through expansion or growth previous to division. The fate of the nucleoli was not discovered. They have generally disappeared by the time the chromatin coil has segmented. In some cases they appear quite vacuolate (*fig. 11*), in others of the same consistency throughout (*figs. 15, 16*). It will be seen from an examination of the figures that the spindle is bipolar from the first. It arises as two closely applied caps on opposite sides of the nucleus at the summits of which are well defined centrospheres. These centrospheres gradually extend outwards, drawing the spindle into a sharp pointed bipolar structure. In the case of the onion, therefore, it is an impossibility for the spindle to arise by an aggregation of many cytoplasmic radiations which first form multipolar structures passing out on all sides of the nucleus, as has been described by Mottier, Osterhout, and others. The spindle is so sharply defined from the very first that it can be traced step by step in all its stages of development, its limits always appearing with proper staining very distinct and sharply differentiated from the cytoplasm. In some cases, where the cells are very flat in

longitudinal diameter, the spindle also appears very much flattened (*fig. 21*). Were such a spindle sectioned it could easily give the appearance of a multipolar structure. No such cases, however, were found. If the spindle extended clear across the cell so that it touched the opposite walls it might give the appearance of the threads ending in the cell wall. Although the nature and origin of the spindles in *figs. 19*, *21* and *22* are exactly the same, there is a striking difference in their shape, and very suggestive of how the shape of the cell may influence the appearance of the karyokinetic figures. The same is evident from a comparison of *figs. 6* and *10*. Often the two poles of a spindle are not 180° apart. This is caused no doubt by the centrospheres not becoming exactly opposed (*fig. 19*). After the nuclear membrane has disappeared the V-shaped chromosomes are gradually drawn down into the equatorial plane, with their heads toward the center, until they form quite a symmetrical figure (*figs. 22–26*). The centrosome usually does not divide until after the formation of the mother star, but sometimes the division may occur earlier (*fig. 23*). The longitudinal splitting of the chromosomes takes place about or during the time of the formation of the mother star (*figs. 27*, *28*). When the cell is very long and narrow there does not appear to be a typical mother star formed (*fig. 24*). In such cases the chromosomes do not appear to be drawn symmetrically into the equatorial plane. The chromosomes appeared quite homogeneous throughout, nothing being visible having the appearance of chromatin granules. It was not possible to tell exactly how the chromosomes are arranged on the achromatic spindle threads, but the threads seemed to be in bundles running continuously from one pole to another, ending in the hyaline area of the centrosphere and having the chromosomes attached by their heads (*figs. 28–30*).

When the chromosomes have been brought into the equatorial plane, and longitudinal splitting is complete, the daughter chromosomes are gradually pulled apart, and the central spindle begins to appear between them in the equatorial region. Some-

times the figures of the metakinesis stage are remarkable for their symmetrical development (*figs. 29, 30*). Such symmetry could not be present were the two ends of the spindle formed at haphazard from variable numbers of irregular smaller elements. The centrosomes usually divide during metakinesis (*figs. 31, 32*). By the time the daughter chromosomes have arranged themselves around the poles, the centrospheres, as a general rule, have divided and the radiations show more prominently than in the earlier stages (*fig. 23*). The chromosomes now begin to contract and the free ends turn inwards, while at the same time the threads of the central spindle become thickened and stain much deeper than before. The polar radiations also become more widely separated because of the outward pressure exerted by the chromosomes (*fig. 34*). At the time when the chromosomes are curving inward the central spindle threads begin to bulge outwards, and the cell plate is formed from the center, appearing at first as granular thickenings in the spindle threads. In this stage the centrospheres often appear still united but containing a double centrosome (*figs. 35, 36*). In *fig. 37* only one centrosphere is visible at the upper pole, the other one lying immediately beneath the one in view.

The central spindle continues to bulge outward and the cell plate becomes larger, until finally when it reaches the cell walls the spindle has a very flattened appearance (*figs. 38, 39*). The spindle threads continue to stain very dark at the center until the cell plate is complete. What the cause of this dark staining may be was not discovered. It was probably due to the presence of various materials in the thickened spindle threads which are used in the formation of the cell wall. It is not easy to understand how the threads of the central spindle extend outward until they are sometimes almost doubled on themselves. But whatever the direct cause, they are considerably longer than they were at first. The central spindle threads disappear as soon as the cell wall is well formed, being absent in the center while they are still prominent in the outer regions (*fig. 39*). As soon as the cell wall is complete the threads disappear

entirely. Whether they remain in the cytoplasm, or are withdrawn into the nucleus, or furnish part of the material for the nuclear membrane, are all matters of mere conjecture. The nucleoli begin to appear a little before the time when the cell wall has been completely formed.

Fig. 41 is an interesting case in that it shows the centrosome not yet divided in a very late stage. This body appears as a long, black, rod-like body forming a slender dumb-bell. The chromatin bands seem to be distributed again or spread out in a fine network, and the nucleus continues to swell out and become more rounded until the complete resting stage is again attained. The depression formed at the pole, however, remains, and in this there can often be seen exceedingly distinct centrospheres. Although the cases in the resting condition are not numerous where these bodies appear very distinct, yet in such cases as *fig. 42* there can be no doubt of the continuance of the centrospheres into the resting stage of the nucleus. In the example given in *fig. 42* the whole cell is remarkably clear and free from granules, the two prominent bodies lying alone in the polar depression. To claim that these bodies are not centrospheres would be exceedingly dogmatic, and the only recourse left would be to name and describe two new organs of the cell which have the same appearance and occupy the same position as do real centrospheres.

The general process of karyokinesis for the onion root may be summarized as follows:

I. PROPHASE.

1. The division begins with the separation of the centrospheres, and when these have moved apart nearly 180° the incept of the achromatic spindle appears, forming two dome-shaped projections on opposite sides of the nucleus, at the summits of which the centrospheres are situated, forming the poles around which are cytoplasmic radiations. At the same time the chromatin network is transformed into a continuous ribbon or spirem producing the figure known as the *close mother skein* (*figs. 2–10*).

2. The continuous spirem shortens and thickens and is looped into a definite number of loops, the heads of which, in typical cases, point toward the two poles of the spindle. The nucleoli and nuclear membrane disappear and the dome-shaped spindle becomes more pointed by the outward extension of the poles. This stage ends with the breaking of the chromatin loops into separate chromosomes, and it may appropriately be called the *looped mother skein* (*figs. 11–19*).

II. METAPHASE.

3. After the nuclear membrane disappears, the separate chromosomes are drawn down, with their heads toward the center, into the equatorial plane, while the spindle continues to become more pointed (*figs. 20–25*). This constitutes the *loose mother skein* stage.

4. When the chromosomes have come into the equatorial plane, there is a pause resulting from the seeming pull of the spindle fibers in opposite directions, which holds the chromosomes rigidly until the longitudinal splitting of the chromosomes is complete, when separation of the daughter chromosomes begins (*figs. 26–28*). This constitutes the *mother star* stage.

III. ANAPHASE.

5. After the longitudinal segmentation of the chromosomes which, as a general rule, does not begin until the chromosomes are in the equatorial plane, the daughter chromosomes are gradually pulled apart, the separation beginning at the heads of the loops. The centrosomes usually divide during this stage, though in some cases the division may be considerably earlier. This stage is appropriately known as *metakinesis* (*figs. 29–31*).

6. The daughter chromosomes having been completely pulled apart, now travel to the poles and arrange themselves in star-shaped figures around the poles, while the central spindle appears between the two stars. The radiations around the centrospheres, which now contain two separate centrosomes, become more prominent (*figs. 32, 33*). This is the *daughter star* stage.

IV. TELOPHASE.

7. The chromosomes having oriented themselves around the poles, now begin to contract, becoming wavy in outline, and the free ends curve inward. The threads of the central spindle begin to thicken preparatory to the formation of the cell plate. In the center of each thickened thread a granule appears, these being formed first in the central strands, and as the spindle bulges outward the cell plate gradually enlarges until it reaches the surrounding cell wall. In the meantime the nucleoli begin to appear in the daughter nuclei. This stage may be called the *loose daughter skein*, and may be considered to end when the cell plate is complete (*figs. 34–39*).

8. After the daughter cells are completely separated by the new cell wall the threads of the central spindle disappear, and the daughter nuclei appear with complete nuclear membranes. The chromosomes begin to be transformed again into the chromatin network; the radiations disappear from around the centrospheres, which have now usually divided completely into two separate bodies; and the two daughter nuclei in the meantime expand and take on a more spherical form until they enter again into the resting stage (*figs. 40–42*). This stage may be known as the *close daughter skein.*

Fig. 1 may be taken as a typical nucleus in the tissue beyond the growing point, showing in detail the actual arrangement of the chromatin network, chromatin granules, and nucleolus. *Fig. 2* represents a typical cell in the active part of the meristematic region.

To illustrate the normal order of karyokinesis, the following figures may be taken as a complete series: *2, 4, 5, 8, 11, 13, 16, 17; 20, 22, 25; 26, 27, 29, 30, 32, 33; 34, 35, 36, 38; 39, 40, 42.* A briefer series may be represented by the following: *2, 5, 8, 13, 17, 20, 25, 26, 27, 30, 32, 33, 34, 36, 39, 40, 42.*

SAGITTARIA VARIABILIS.

The anilin-safranin, iron-alum-haematoxylin combination was also tried on dividing pollen mother cells of Sagittaria. The results were even more striking than in the onion. In the mother star stage the centrosomes at the poles look like large black

spherical granules, but the attraction sphere is usually not very well differentiated. The poles usually lie very close to the wall of the cell, giving little or no room for polar radiations (*figs. 43, 44*). The figures drawn are not exceptional cases, but scores of similar figures can be seen in a single section across the flower bud. From a careful estimate, I have a single slide which will show several hundred figures of the same nature as those given. A careful search was again made for multipolar spindles, and in this material they are frequently seen. This is not at all surprising, however, and is exactly what must necessarily follow the sectioning of tissues where the spindles do not all lie in the same plane. Especially in thin sections is the pole often cut away, giving the appearance that the spindle does not end in a single point. Since the spindle threads in Sagittaria pollen mother cells are massed into definite bundles, any injury to the spindle will produce a multipolar spindle. Such a case is shown in *fig. 45*, where the cell has been crushed at one end, producing four apparently separate spindles on the lower side, while the upper end is practically intact. The centrospheres appear at the two original poles. At the present time, in all the material examined by the writer, multipolar spindles seem due entirely to two causes: first, to pathological conditions; and second, to injuries of the spindle produced by improper manipulation in preparing the sections. The latter may be due to a variety of causes. Among the more common of these may be mentioned improper killing and treatment of material, sectioning the cells into such thin slices that the poles are entirely lost or injured, cutting off the poles from the spindles which lie diagonally to the plane of the section, and finally, injury by crushing the cells in such a manner that the spindle is spread out and torn.

COLUMBUS, O.

EXPLANATION OF PLATES XXI, XXII.

The drawings have been reduced three-eighths of their original size. They were drawn with an Abbé camera, and except in one instance combinations of Zeiss and Bausch and Lomb oculars and objectives were used.

PLATE XXI.

FIG. 1. A resting nucleus from a cell beyond the growing point. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 18, B. and L. obj. $\frac{1}{12}$.

FIG. 2. Resting cell with centrospheres from the growing point. Anilin-safranin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 3. Cell just before division, stained very deeply. Iron-alum-haematoxylin. Zeiss. oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 4. Nucleus with centrospheres on opposite sides, in early stage of division. Iron-alum-haematoxylin. Zeiss oc. 12, obj. 2^{mm} ap. hom. im.

FIG. 5. Cell with incept of achromatic spindle and centrospheres at the poles. Iron-alum-haematoxylin. Zeiss. oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 6. Long cell with bipolar spindle having sharper ends than usual at this stage. Acid fuchsin, methyl-green. Zeiss. oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 7. Close mother skein with no spindle visible, but with radiations at the two ends of the nucleus. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 8. Cell with centrospheres and granular zones outside of the polar radiations. Iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 9. Cell with late separation of centrospheres and precocious division of the centrosomes. Delicate radiations around the centrospheres. Anilin-safranin, gentian-violet. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 10. Nucleus with very flat dome-shaped spindle and cytoplasmic radiations or streams. Acid fuchsin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 11. Cell with centrosomes and distinct coarse radiations. Anilin-safranin, gentian-violet. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 12. Early dome-shaped spindle with no centrosomes visible. Iron-alum-haematoxylin. Zeiss oc. 12, obj. 2^{mm} ap. hom. im.

FIG. 13. Looped mother skein showing radiations around the poles of the dome-shaped spindle. Acid fuchsin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

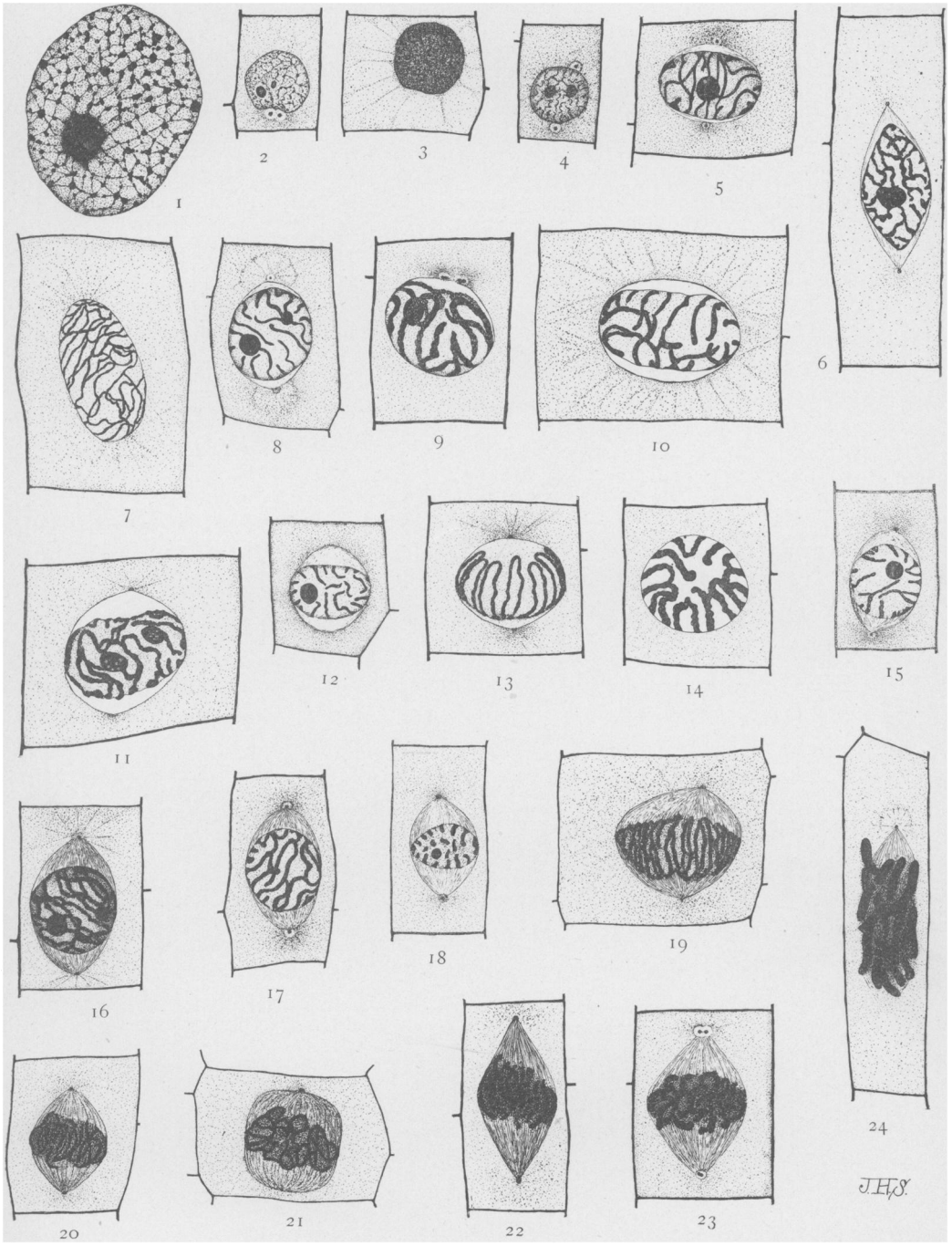
FIG. 14. End view of looped mother skein. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 15. Dome-shaped spindle with centrospheres. Anilin-safranin, gentian-violet. Zeiss oc. 12, obj. 2^{mm} ap. hom. im.

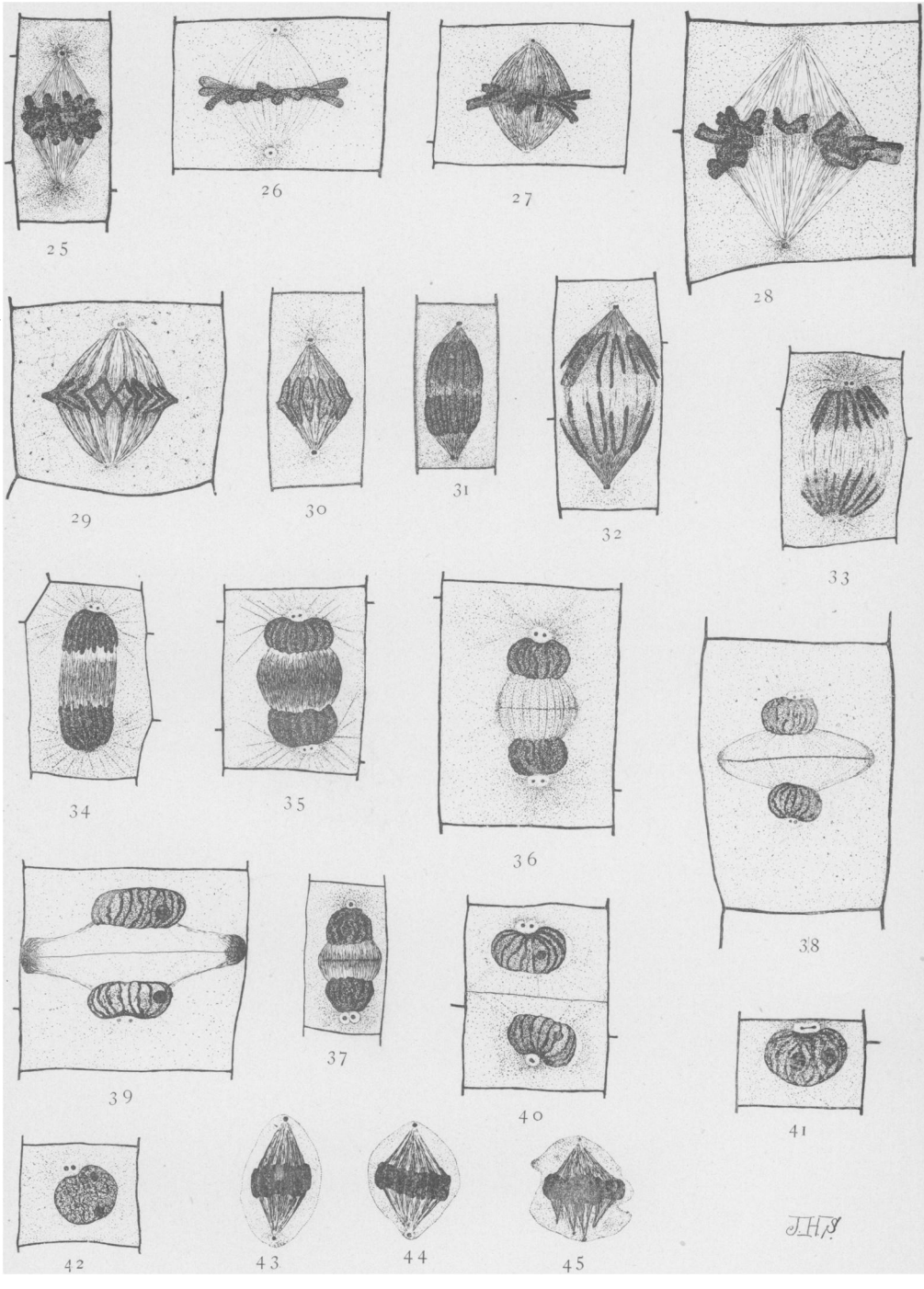
FIG. 16. Dome-shaped spindle becoming pointed, with prominent radiations around the poles. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 17. Dome-shaped spindle with prominent centrospheres. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 18. Spindle becoming pointed. Anilin-safranin, gentian-violet. Zeiss oc. 12, obj. 2^{mm} ap. hom. im.



SCHAFFNER on KARYOKINESIS.



SCHAFFNER on KARYOKINESIS.

FIG. 19. One-sided spindle with looped spirem. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 20. Loose mother skein with centrosomes at the poles of the spindle. Iron-alum-haematoxylin. Zeiss oc. 12, obj. 2^{mm} ap. hom. im.

FIG. 21. A flat cell with a very flat spindle. One end shows a centrosome. Anilin-safranin, picric nigrosin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 22. Spindle much as in *fig. 20*, but more pointed. Anilin-safranin, gentian-violet. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 23. Loose mother skein with large centrospheres in which the centrosomes have divided earlier than usual. Anilin-safranin, gentian-violet. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 24. Long cell showing a sharp-pointed spindle with centrosphere and an outer granular zone in the radiations. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

PLATE XXII.

FIG. 25. Loose mother skein showing spindle with typical centrospheres and radiations. Iron-tannin, safranin. Zeiss oc 12, B. and L. obj. $\frac{1}{12}$.

FIG. 26. Typical mother star. Anilin-safranin, gentian-violet, Gram's iodin potassium iodid. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 27. Segmented mother star. Acid fuchsin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 28. Segmented mother star from large cells of central strand. One end shows a very marked centrosome, the other none. Anilin-safranin, gentian-violet. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 29. Early stage of metakinesis with spindle still somewhat dome-shaped because of the flatness of the cell. Centrosomes divided. Anilin-safranin, picric nigrosin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 30. Metakinesis stage showing radiations around the poles. Anilin-safranin, gentian-violet. Zeiss oc. 12, obj. 2^{mm} ap. hom. im.

FIG. 31. Last stage of metakinesis; centrosomes dividing. Anilin-safranin, gentian-violet. Zeiss oc. 12, obj. 2^{mm} ap. hom. im.

FIG. 32. Daughter star stage; centrosomes dividing. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 33. Daughter star. Prominent radiations around the poles. Anilin-safranin, gentian-violet. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 34. Beginning of loose daughter skein. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 35. Loose daughter skein with coarse radiations. Gentian-violet, eosin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 36. Loose daughter skein with early stage of cell plate and prominent centrospheres. Anilin-safranin, gentian-violet, Gram's iodin potassium iodid. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 37. Loose daughter skein with prominent centrospheres. At the upper pole one centrosphere is hidden. Iron-tannin, anilin-safranin. Reichert oc. 12, Leitz obj. $\frac{1}{12}$.

FIG. 38. Loose daughter skein with large cell plate. Anilin-safranin, picric nigrosin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 39. Close daughter skein with cell plate about complete. Acid fuchsin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 40. Close daughter skein with cell plate complete. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 41. Close daughter skein with dumb-bell-shaped centrosome delayed in division. Anilin-safranin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 42. Resting daughter cell with remarkably distinct centrospheres. Iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 43. *Sagittaria variabilis*. Microspore grandmother cell showing spindle with large centrosomes. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.

FIG. 44. Same as *fig. 43*.

FIG. 45. *Sagittaria variabilis*. Microspore grandmother cell somewhat crushed, resulting in a distinct multipolar spindle. Centrosomes still visible. Anilin-safranin, iron-alum-haematoxylin. Zeiss oc. 12, B. and L. obj. $\frac{1}{12}$.